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NUTRITION REVIEWS

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CHANGES IN NUTRITIVE VALUE OF FOOD FATS DURING PROCESSING AND COOKING

The importance of fat in the American diet is now recognized by all workers in the science of nutrition. A quantitative evidence of this fact is the annual per capita consumption of fats in the USA which is presently close to 100 pounds per year, made up roughly of 45 pounds of visible fats (butter, margarine, lard, shortening, vegetable oils) and 55 pounds consumed as so-called invisible fat, *i.e.*, fat which is intimately associated with, and hence not separable from, other components of these foods.

The fats are primarily triglycerides of mixtures of saturated and unsaturated fatty acids, the nature of the mixture depending on the fat in question. Generally, the common animal fats in our foods tend to be somewhat more saturated than the vegetable but there are exceptions both ways. The principal so-called visible food fats in the American diet are presently lard and butter of the animal fats, and corn, cottonseed, peanut and soybean oils of the vegetable fats. Of the latter, large amounts of cottonseed oil and most of the soybean oil used as food fat are hydrogenated in varying degrees and used in the formulation of shortenings and margarines where they come into present competition with lard and butter.

Of the several contributions of fats to the human diet, the only one which is specific is the contribution of the essential fatty acids (EFA), more particularly linoleic and arachidonic acids. These have been proved to be essential to many species of animals, and most workers accept their essentiality in man. The EFA are polyunsaturated acids, *i.e.*, they contain two or more double bonds per fatty acid carbon chain. Because of this unsaturation they are the most sensitive of the fatty acids to oxidation and any other type of chemical change. Indeed, any chemical change in the EFA impairs their specific biological value.

The best sources of linoleic acid are the so-called semi-drying oils, namely, corn, cottonseed and soybean oils which readily oxidize in air, even at ordinary temperatures.

Recent work in several laboratories has shown that the EFA, or perhaps the total content of unsaturated acids, may have some effect in lowering the blood cholesterol level, allegedly a factor in the development of atherosclerosis. Any treatment of fat which seriously alters its EFA or total unsaturated fatty acid content, or which would convert these acids to compounds deleterious to health, should be critically studied and evaluated in long range relation to the health of the American people.

Technologically, most food fats in preparation for table use or in the formulation of food products are put through various types of refinement designed to remove unwanted material. These processes remove natural antioxidants so that, when food fats are to be stored before use, it is necessary to add natural or synthetic antioxidants to extend shelf life. It is not likely that significant alterations in the triglycerides or in the fatty acids occur during the usual refining processes. Furthermore, processing procedures involving interchange of fatty acids within or between glyceride molecules, as is occasionally practiced, is unlikely to involve questionable alterations in the fatty acids themselves.

Fats may be exposed to two types of treatment during commercial processing which may seriously alter the fatty acids and the glycerides and are presently under

serious scrutiny as possible health hazards; first, exposure to high temperatures with or without oxygen which may be encountered in the home kitchen, in restaurants and other eating establishments and in industry where deep fat frying is now so much in vogue, and, second, hydrogenation, a process employed exclusively by the fat industries. Both types of treatment will be briefly discussed.

Generally speaking, heating fats below 200° C, in the absence of oxygen for a reasonable length of time results in no important chemical change. On the other hand, temperatures above 250° C may induce important polymeric changes. Even at ordinary temperatures in air, oxidation can occur with the formation of hydroperoxides which may of themselves be toxic (R. T. Holman and S. I. Greenberg, *J. Am. Oil Chem. Soc.* **35,** *707 (1958)*). Peroxidation involves addition of a molecule of oxygen to a methylene group adjacent to a double bond. The resulting hydroperoxide can either decompose with rupture of the chain, which is likely to be innocuous, or it can rearrange to give hydroxylated compounds and eventually polymers.

The feeding of oxidatively rancid fats may produce impairment of vitamin values either by destroying the vitamin as in the case of vitamin A, or by inhibiting its metabolic action as has been shown to be the case with some of the B vitamins.

Technologically, food fats of the visible type are not ordinarily heated to temperatures above 200° C. If, on the other hand, the fat is seriously overheated either intentionally or accidentally, *i.e.*, at temperatures above 250° C, the unsaturated acids may form polymers between themselves or between glyceride molecules or may internally isomerize. The resulting products may not be digested by lipase and hence may not be absorbed, or, if absorbed, may be actually toxic. Exposure to excessive heat may occur in the kitchen in the course of frying meat, deep fat frying or during broiling over the open grill. If actual charring occurs, tars may be formed, as well as acrolein, an irritant aldehyde decomposition product of glycerol.

Although claims have been made in the lay press that broiling over charcoal grills is a dangerous procedure, it does not seem likely to the writer, and certainly has not been proved in controlled experiments, that the average American is endangered by occasional exposure to the products of this type of cooking. If too much destruction of this type were to occur, the product would be unacceptable from the flavor standpoint.

The deleterious effects of thermal polymerization have been amply demonstrated by data in rat feeding experiments from several laboratories, of which the following have been selected for brief review.

E. W. Crampton and co-workers (*J. Nutrition* **43,** *431, 533 (1951)*; **44,** *177 (1952)*; **49,** *333 (1953)*; **60,** *13 (1956)* and **62,** *341 (1957)*) exposed linseed, rapeseed, corn, soybean and herring oils to temperatures of 275° C in a stream of CO_2. Oils thus treated in the absence of oxygen caused depression of appetite and growth when fed to rats at the 10 to 20 per cent level. These effects were shown not to be due to peroxidation or to lack of vitamin E. The ethyl esters were prepared from thermally polymerized linseed oil and separated into three fractions: unchanged esters with normal nutritive behavior, cyclized esters which were digestible but toxic, and polymerized esters which were not digestible or absorbed but were otherwise non-toxic. The toxic fractions of soybean and safflower oils contained high proportions of "difficultly conjugatable diene cis isomers of cyclic structure".

H. Kaunitz and co-workers (*J. Nutrititon* **55,** *577 (1955)*; **60,** *237 (1956)*; *Fed. Proc.* **14,** *408 (1955)*; *J. Am. Oil Chem. Soc.* **33,** *1 (1956)*) aerated lard at 100° C for 200 hours and found it to become only mildly toxic, whereas cottonseed oil became severely so. Molecular distillation of the heated fat up to 275° C gave fractions

ranging from non-toxic to mildly toxic. The residues at this temperature (17 per cent in the case of lard and 41 per cent in the case of cottonseed oil) were severely toxic. These authors feel that oxidation products are not the main sources of toxicity. The polymeric residues from autoxidized cottonseed oil exerted a greater growth-depressant effect than those from lard, which in turn were greater than those from a hydrogenated cottonseed oil used for commercial deep fat frying.

These experiments involve treatment which is drastic only in the element of time and are applicable practically only when fat in deep frying operations is used too long.

A third series of investigations on heated fats has been reported by F. A. Kummerow and co-workers. Corn oil, butter and margarine fats were aerated for periods up to 24 hours at 190 to 200° C. Under these conditions corn oil developed a marked growth depressing action, margarine fat only a slight action of this kind, and butter none.

In a study of the chemical changes taking place during thermal oxidation of corn oil (O. C. Johnson and Kummerow, *J. Am. Oil Chem. Soc.* **34,** *407* (*1957*)), it was shown that there was a fall in iodine value and an increase in viscosity with only a moderate increase in peroxide value. Later, L. A. Witting, T. Nishida, Johnson and Kummerow (*Ibid.* **34,** *421* (*1957*)) reported that dietary riboflavin and pyridoxine levels were factors in the nutritional behavior of thermally oxidized fats. Riboflavin seemed to be of special value to rats in coping nutritionally with these fats.

It was still further shown (Johnson, E. G. Perkins, Sugai and Kummerow, *J. Am. Oil Chem. Soc.* **34,** *594* (*1957*)) in a study involving treatment of corn oil, hydrogenated cottonseed oil, olive oil and oleo oil under thermal oxidation at 180° for 24 hours, that most of the loss of nutritional value was at the expense of linoleic acid and was proportional to the original linoleic acid content. Thermally oxidized corn oil was more slowly hydrolyzed by pancreatic lipase than the original oil, although both appeared to be equally well absorbed.

Still another paper from this laboratory (Perkins and Kummerow, *J. Nutrition* **68,** *101* (*1959*)) described the nutritional effects of the polymers from corn oil thermally oxidized at 200° C for 48 hours. The fatty acids were recovered and subjected to urea adduct fractionation and molecular distillation. Rats were fed on a diet containing 2 per cent cottonseed oil and 12 per cent of the test fat or fatty acid fraction in question. All animals fed the non-distillable residues from the non-urea adduct forming fatty acids from the oxidized oil died within seven days. Dilution of these acids with an equal volume of fresh corn oil fatty acids permitted the rats to survive for 21 days, but counteracted only partially the growth depressing effect. In work in press several polymer fractions of molecular weights from 692 to 1600 were isolated. These had a high content of oxygen, present as hydroxyl and carbonyl groups, and were linked together in a non-cyclic structure.

It is clear from the above work that thermal polymerization of fats and oils, either with or without oxidation, forms products which seriously impair the nutritive values of the oils in question and may even be actually toxic.

Deep fat frying operations are employed by the potato chip industry and by others in the manufacture of french fries and other potato products as well as many other foodstuffs for the frozen food industry. Some evidence has been reported which claims to show no serious damage as a result of such operations. D. Melnick (*J. Am. Oil Chem. Soc.* **34,** *578* (*1957*)) in reviewing the preceding work, feels that in general the results are irrelevant to commercial operations. In an extended survey of oils used in the manufacture of potato chips, in which a large number of processors cooperated, he concluded there was little or no evidence of

serious oxidation or polymerization, but there were decreases in iodine value amounting usually to less than two units. Small decreases in linoleic acid content were also noted. In this work the oils under study were corn, cottonseed (cooking and winterized) lightly hydrogenated cottonseed oil, shortening and shortening blend.

In another paper (*J. Am. Oil Chem. Soc.* **35,** *271 (1958)*), Melnick could find no evidence for the presence of polymers in oils used in making potato chips, but did note a decrease in iodine value less than 3.0 per cent in 95 per cent of the operations.

Most recently K. W. Keane, G. A. Jacobson and C. H. Krieger (*J. Nutrition* **68,** *57 (1959)*) have reported no toxicity in a specimen of hydrogenated cottonseed oil used in deep fat frying and, amazingly, an actual increase in unsaturation with use in processing over many days.

From the evidence presented above, it has been definitely shown that thermal polymerization of fats results in products which are either not absorbed (reduction in digestibility) or are actually toxic and that these changes occur most readily in the more unsaturated fats, but it should be pointed out that the conditions under which the changes occurred experimentally are far more drastic than ordinarily occur under practical conditions. Certainly sensible precautions are in order in the home, in restaurants and drive-ins not to overheat fats and not to use them too long in deep fat frying, whereby toxic products might be produced. Frankly, it is certainly not proved that the occasional use of fats thus treated will have any permanent damaging effects on health.

In industry, on the other hand, it would seem to be in order to take sensible steps to avoid processing conditions involving thermal oxidation and thermal polymerization, and under no condition to allow the marketing of products containing fats thus seriously damaged. The use of polymerized fats as an additive to food fats should be seriously questioned.

While it is apparent from the preceding discussion that serious chemical alteration of food fats by ordinary processing, and by oxidation, and/or polymerization is the case only occasionally, and perhaps most often by accident, the situation is entirely different for commercial hydrogenation of fats resulting in profound changes in chemical composition. The amounts of fat hydrogenated are enormous. A recent estimate indicates that something like 440 million pounds of cottonseed oil and 1200 million pounds of soybean oil are hydrogenated in this country annually, the resulting products being used largely in the formulation of margarines and shortenings.

Hydrogenation involves several types of chemical change. These are: (1) Addition of hydrogen to double bonds forming more saturated compounds of higher melting point. In illustration, it may be cited that linoleic acid with two double bonds melts at −5° C, oleic acid with one double bond at 13° and stearic acid, a saturated acid, at 69°. (2) Shift of double bonds. Thus, oleic acid with one double bond between the ninth and tenth carbon atoms of the 18-carbon chain may conceivably shift either towards the carboxyl to the 8-position or away from the carboxyl to the 10-position. Both products are so-called iso-oleic acids. (3) Formation of trans acids, which is another form of isomerization, also tends to raise the melting point. (4) Formation of conjugated acids. Thus linoleic acid with two double bonds separated by a methylene group (CH_2) may rearrange to a conjugated cis in which the double bonds are adjacent. In nature, acids of this type occur infrequently.

The total result of these several types of changes is to alter almost completely the unsaturated acids of the original fat. For example, if cottonseed oil with an iodine value of 110 and containing 50 per cent of linoleic acid is hydrogenated partially to an iodine value of 75 to 80, the resulting product may contain less than 10 per cent,

which is very close to 90 per cent destruction of this EFA (B. Sreenivasan and J. B. Brown, *J. Am. Oil Chem. Soc.* **33,** *521 (1956)*). In addition, the product may contain from 25 to 40 per cent of trans acids and many other unnatural isomers (Mabrouk and Brown, *Ibid.* **33,** *98 (1956)*).

Perhaps the most important change in nutritive value brought out by commercial hydrogenation is the enormous destruction of an essential nutrient. A rough calculation of this loss of EFA from the amounts hydrogenated as stated above is between 850 million to one billion pounds, or 6 to 7 pounds per person per year. This is almost one half the total availability of the EFA in our visible food fats.

The question remains concerning the nutritive value of the numerous isomers in hydrogenated fats. Melnick (*J. Am. Oil Chem. Soc.* **31,** *63 (1954)*) claims that the iso-acids are not antimetabolites to oleic acid and behave normally in metabolism. P. V. Johnston, Johnson and Kummerow (*J. Nutrition* **65,** *13 (1958)*) reported that the trans isomers, when fed to rats, are deposited in rat tissues and seem to be efficiently metabolized. They did not appear to inhibit growth. The unnatural isomers formed during hydrogenation are deposited in human tissues (*Science* **126,** *698 (1957)*), and may be solely of dietary origin (*Proc. Soc. Exp. Biol. Med.* **99,** *735 (1958)*). In unpublished results from our laboratory, trans acids have been found in human depot, liver and milk fats in amounts up to 6 per cent.

R. B. Alfin-Slater and co-workers (*J. Nutrition* **63,** *241 (1957)*) fed rats for 46 generations on a selectively hydrogenated fat at the 9.24 per cent level and have found no evidence of deleterious effects, stating that it was "fully digestible, harmless and of full nutritional value." On the other hand, Holman and E. Aaes-Jorgensen have shown deleterious effects on the testes from feeding trans acids to rats (*Proc. Soc. Exp. Biol. Med.* **93,** *175 (1956)*).

Generally speaking, there is no evidence that the extensive use of hydrogenated food fats in this country is attended by any serious health hazard. As stated previously, the principal proved consequence of hydrogenating fat, in relation to nutritional values, is the tremendous reduction in EFA content. If the EFA, or the total unsaturated acids, are an important factor in lowering blood cholesterol and if the lower cholesterol level is an advantage in preventing atherosclerosis (alleged but not proved), developing of processing methods designed to retain these acids in dietary fats would be a laudable objective of the fat industries. In any event it would seem wise to continue to study the effects of long time use of hydrogenated fats in human nutrition.

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INBORN ERRORS OF METABOLISM

Inborn errors of metabolism are inherited defects in the chemistry of the body. These take many forms, some common, some strange and grotesque.

The strange appearance of Noah, who was born an albino, (A. Sorsby, *Brit. Med. J.* **2,** *1587 (1958)*) caused consternation to his father, Lamech, for his "flesh . . . was white as snow, and red as a rose; the hair of (his) head was white like wool, and long; and (his) eyes were beautiful." According to N. Avigad and Y. Yadin (*A Genesis Apocryphon. The Magnes Press, Jerusalem, p. 40 (1956)*), Lamech's wife was either his

sister or his first cousin. This consanguinity may have accounted for the first recorded instance of an inborn error of metabolism. However, it was not until A. E. Garrod (Lancet **II**, *1*, *73*, *142* (*1908*)) so classified them (albinism, alkaptonuria, cystinuria and pentosuria) that some measure of understanding began. Indeed, he concluded that they were genetically determined and supposed that they were due to lack of enzymes essential for normal metabolism.

Since then, new metabolic defects have been detected, recognized or suspected until more than 30 are known. But why concern ourselves with rare diseases which affect so few of our population? We are concerned because these "experiments of nature" reveal pathways of normal as well as abnormal metabolism, disclose the mode of action of certain enzyme systems and provide invaluable genetic information about mankind.

To many students of medicine, chemistry and biology these disorders are bewildering. Consequently, it is refreshing to find simple clarity and logic in N. Zöllner's discussion of "Inborn Errors of Metabolism" (*J. Chron. Dis.* **10**, *6*, (*1959*)).

Most of these metabolic errors are due to a simple block of a metabolic pathway. The normal pathway may be represented by A-B-C-D-E-F, and an enzyme is assumed to be necessary for each step of the conversion. In this theoretical scheme one enzyme, for example that which converts D to E, is absent or deficient. Several possibilities now become evident. First, an uncompensated block may exist which leads to a surplus of D, but a paucity of E and F. Secondly, a compensated block may occur by means of an alternate pathway, allowing D to be converted into new substances G and H. These may be harmless or toxic. Nevertheless a deficiency of E and F persists. Finally, another form of compensated block may occur if an alternate means for manufacture of E and F exists. In this instance, D accumulates excessively but may do no harm.

Metabolic blocks are understood best by considering them to be a result of a deficient enzyme. A reasonable explanation of their occurrence is as follows. A mutation occurs in a gene of a person and this change is inherited by his progeny. Genes contain desoxyribonucleic acid and are able to transfer their specific characteristics to ribonucleic acid, the cytoplasmic matrix of all cells. This matrix manufactures other proteins, some of which will serve as enzymes, some as transport proteins, and some as cellular membranes. Hence any defect of an inherited gene will be transmitted to all cells of the body.

In many instances this may have no apparent effect upon growth and metabolism, but in others it may lead to gross disturbance. The simplest, a metabolic block, results in biochemical abnormalities. The second, a compensated block, can produce new substances such as abnormal hemoglobins (S, C, D, and E). The third example of a metabolic block may be compensated to some degree by other systems. For example, in diabetes mellitus glucose is not metabolized properly, but energy is furnished (imperfectly) by utilization of lipids. The accumulation of excessive glucose is of little basic importance.

Several examples of metabolic errors fit into this scheme quite well and provide fragmentary "laws" which are useful in interpreting other disorders. Galactosemia is an enzymatic defect which results in injury to the central nervous system, cataract and hepatic disease of newborn infants if unrecognized. However, if these infants are fed foods not containing lactose, their development will be normal. Here then is a true example of one man's food becoming another's poison. It exemplifies the fact that a genetically determined disease may not become apparent until environmental factors provide the substance which

cannot be metabolized properly. If a noxious substance accumulates before the block, then its precursors must be avoided. (One wonders about the latent period in patients who will develop diabetes, gout, Wilson's disease, adrenal virilism and others.)

Defects of transport proteins may be common. In cystinuria, the concentration of this amino acid in the blood is normal, yet the rate of urinary excretion is high. In this instance renal tubular reabsorption of diamino acids is faulty, resulting in excretion of large quantities of cystine, lysine, arginine, and ornithine. Since cystine is less soluble, it crystallizes in the kidneys leading to clinical disease.

Wilson's disease is another example of a faulty transport mechanism. At least part of the defect depends upon a deficiency of ceruloplasm, which carries copper in the blood. Although concentrations of copper in the blood are low, this metal accumulates in the brain, liver and other organs with accompanying scarring and destruction of tissue.

Gout has been much studied, yet remains a puzzle. Zöllner believes that metabolic blocks must be "defects toward minus", that is, an enzyme may be absent or faulty but it cannot be overactive. Applying this principle to gout, he reasoned that uric acid, which is an end product of purine metabolism, could not accumulate before a block. Other studies have shown no evidence for defective renal tubular handling of uric acid. Hence he reasoned that uric acid could be produced in excess only as a result of a compensated block of some other metabolic pathway which formed uric acid instead of the intended product.

In two familial lipidoses, hypercholesterólemia and hyperlipemia, one finds abnormal quantities of not one but several biochemically unrelated lipids in the blood. To explain this, Zöllner calls attention to the intersolubility of lipids which results in simultaneous transport of phospholipids, cholesterol and others.

Certain metabolic errors are capable of producing physical distortions of the body. The storage diseases (thesauroses) are examples of a lack of an enzyme. Precursor substances, if insoluble, will accumulate in the involved cells producing enlargement and distortion. Not so easily explained are the anatomical freaks such as gargoylism and arachnodactyly. In the former, mucopolysaccharides are considered to be improperly produced, while in the latter the urine contains abnormal quantities of hydroxyproline, a substance necessary for growth of connective tissues.

Atypical or exaggerated responses to drugs may be metabolic defects. Reactions to quinidine (hemolysis and thrombocytopenia) or the extreme sensitivity of Mongols to atropine may be examples of latent defects triggered by administration of a substrate.

The genetic features of inborn errors of metabolism are fascinating. Apparently Noah was the first recorded human albino and may have handed this defect down to modern man. A recent mutation must have occurred in Sweden for Waldenstrom was able to trace 150 of 230 cases of porphyria to a single ancestor born in 1672.

Phenocopies of diseases which are genetically determined in man may be produced in animals by feeding certain substances. Examples are the effect of feeding cholesterol to rabbits which results in a form of atherosclerosis, or of feeding the sweet pea factor (beta amino propionitrile) to growing rats which produces skeletal and vascular lesions similar to those of Marfan's syndrome. These examples suggest that a single toxin (or a single metabolic defect) can induce wide-spread manifestations of disease. Perhaps we should re-examine the theory of "pleotropism of genes" which implies multiple mutations. Apparently one is sufficient.

These same metabolic diseases may be considered as models of the more general afflictions of mankind. At the same time a detailed understanding of them will provide

better means of therapy such as avoidance of precursors of toxic substances or elimination of accumulated toxic products. Wilson's disease is an example of the latter. The accumulation of copper which results from this defect can be reduced by several methods (BAL, penicillamine, intravenous amino acids or potassium sulfide).

Recognition of inborn defects may lead to anticipation of disease in offspring. Marriage of two heterozygous carriers of the sickle-cell trait probably will lead to offspring with the disease.

The known disorders of metabolism have provided two important pieces of information, namely, these defects are omnicellular and they can be precipitated by environmental factors. Thus they furnish a singular opportunity for study of normal metabolism.

Zöllner has made a successful effort to explain the known facts about inborn errors of metabolism by applying simple logical hypotheses which could fit both recognized and new metabolic defects. His lucid reasoning serves as a contribution to both chemistry and medicine, and his theories are not dogmas but working models subject to change.

ABSORPTION OF LABELED IRON IN INFANTS

The absorption of oral iron by infants under three months of age has been found to be greater than that in older infants and children. The validity of isotope experiments for measuring iron absorption has been questioned.

Isotopically labeled iron has been used for more than a decade to estimate the amount of iron absorbed from the gastrointestinal tract. Several well-established observations have emerged from investigations in adults. (1) The absorption of labeled food iron by normal individuals is of the order of 1 to 10 per cent on intakes of 10 to 100 mg. corresponding to estimates of the daily excretion of iron of about 1 mg. (2) There is a considerable individual variation in iron absorption probably not due to methodologic factors. (3) The fraction of labeled iron absorbed is inversely related to the amount of iron given and is also dependent on previous exposure of the gastrointestinal tract to iron. These observations have been interpreted as implying a "mucosal block". (4) The fraction of labeled iron absorbed has been found to be dependent upon the ionic form in which it is present, and also upon the amount of ascorbic acid or other reducing substances taken at the same time.

L. Garby and S. Sjölin (*Acta paediat.* **48**, *Suppl. 117, p 24 (1959)*) have measured the absorption of tracer doses of iron in infants between ten and 90 days of age on usual infant formulas consisting almost entirely of milk. In most of the experiments the tracer dose was given as iron citrate with the formula. Two microcuries of $iron^{59}$ were incorporated in test doses consisting of 20 to 450 micrograms of iron. These iron intakes can be considered physiologic for infants on such diets. Iron absorption was considered to be the difference between the iron administered and the iron present in the stools. A carmine marker was usually given with the test dose and, in most cases, all the activity appeared about 48 hours later in the stool that was stained with carmine.

The per cent absorption of $iron^{59}$ in the test dose varied between 15 and 96 per cent. There was greater variation between individuals than could be attributed to the probable error of the method itself. There was, as had been expected, a negative correlation between the amount of iron administered and the percentage absorbed. Iron doses of 20 micrograms were associated with an absorption of between 60 and 96 per cent, and the two infants receiving more than 200 micrograms averaged close to 36 per cent. The

decrease in tracer absorption with increasing load was 0.2 per cent per microgram and significantly different from zero.

When the data are plotted against age and compared with similar observations made by J. Schultz and N. J. Smith (*Am. J. Dis. Child* **95,** *109* (*1958*); *Nutrition Reviews* **16,** *170* (*1958*)) with which they are comparable, iron absorption in infants under three months of age fell from approximately 80 per cent in the first month to nearly 30 per cent in the third month. The iron absorption data of Schultz and Smith on normal infants averaged approximately 10 per cent for the ages of three through 57 months, and none of the values were above 20 per cent with the exception of one iron deficient child. Within the data of Schultz and Smith there was also a tendency for absorption to decrease with increasing age, most marked after the age of about two years. Thus it was quite obvious that the absorption of tracer iron from milk was considerably larger during early infancy than in later life.

The only available studies in the newborn are those of L. Oettinger, W. B. Mills and P. F. Hahn (*J. Pediat.* **45,** *302* (*1954*)). These infants received the labeled iron without food and with only 1 microgram of iron as ferrous chloride. In these studies the amount of tracer absorbed was calculated from the amount found in circulating red cells after two to six weeks. The percentage absorption was based on the assumption that all of this very small dose of iron was incorporated into the circulating red cells. Recently this assumption, even for very small amounts of tracer iron, has been questioned. The low absorption found by Oettinger, Mills and Hahn (average 3.2 per cent, range 0.4 to 8.2 per cent) is much lower than that found by Garby and Sjölin in infants between ten days and three months of age.

Garby and Sjölin also point out that the absorption of iron in this age group is considerably larger than figures obtained from chemical balance studies on children of similar age and similar food and iron intake (approximately 50 ± 50 micrograms per day for net retention, A. Wallgren, *Rev. Franc. Pédiat.* **9,** *196* (*1933*)). The authors multiplied the per cent absorption of the test dose by the daily intake and found a mean daily net absorption of 322 ± 80 micrograms per day.

This discrepancy has, in their opinion, two possible explanations. The first is that iron is excreted by children of this age group. Secondly, it is possible that true absorption is not measured by the tracer experiments. Their calculations involve the tacit assumption that the passage of iron across the intestinal mucosa is not associated with an isotopic exchange of iron.

In propounding this second possibility, the authors point out that the transmission of a solute across the biological boundary must, in general, be looked upon as a result of two opposing currents, the net flow being the difference between them. A measurement of flow of isotope in one direction may not reflect the net flow accurately. Isotopic exchange may take place with simultaneous transfer in opposite directions of the labeled and unlabeled particles. This can happen even in the absence of a net flow, although the "mucosal block" clearly suggests that iron absorption by the intestinal mucosa is somewhat of an exception to the general state of biological boundaries. The possibilities of isotope exchange have not yet been studied critically.

The authors suggest that the behavior of labeled iron in experiments where an iron load is given prior to the tracer dose, as well as in experiments on iron depleted individuals, is as compatible with such reaction kinetics (that the iron competes for a limited number of reaction sites) as with the assumption that the intestinal mucosa has a regulating power over iron absorption. It is also possible that the iron given with milk feedings was not representative of the iron in those feedings.

In summary, iron absorption in the age group ten to 90 days was found to be much

greater than that found in the newborn, or in older infants and children, and indicated net retentions several times that found in iron balance studies. It is suggested that isotope exchange without net transfer at the mucosal barrier may occur. These concepts challenge both the "mucosal block" theory, and the validity of isotope experiments in measuring both iron absorption and excretion.

TISSUE ELECTROLYTES AND ALDOSTERONE EXCRETION IN PREGNANCY

During the third trimester of normal pregnancy there is an increased excretion of aldosterone, while in pre-eclampsia lower aldosterone excretion levels are associated with higher concentrations of intracellular sodium in muscle biopsies.

During pregnancy, there are both anatomic and physiologic changes in the maternal organism. Of the many chemical changes noted, considerable interest has been expended in studying salt and water metabolism. During the condition of pre-eclampsia (one of the toxemias of pregnancy) there may be edema present, indicating that the body has an increased quantity of fluid. Since aldosterone, a steroid hormone elaborated by the adrenal cortex, is one of the most potent sodium and fluid retaining hormones in the body, the question arises as to the role of aldosterone in pre-eclampsia.

It has been reported by W. J. Dieckmann and R. E. Pottinger (*Am. J. Obstet. Gynec.* **71,** *596 (1956)*) that the total muscle sodium is increased in normal pregnancy, but reverts to the nonpregnant range in pre-eclampsia. On the other hand, H. J. Tatum (*Report No. 1, Ross Obstetrical Research Conference, p. 21, 1956*) has reported no significant changes in the total muscle sodium when normal and toxemic pregnant patients were compared. E. H. Venning and I. Dyrenfuth (*J. Clin. Endocrinol. Metab.* **16,** *426 (1956)*) noted that there is an increased aldosterone content in the urine during late pregnancy. A C. Barnes and E. J. Quilligan (*Am. J. Obstet. Gynec.* **71,** *670 (1956)*) have reported an increased average amount of sodium retaining corticoid (aldosterone) in the urine of patients with pre-eclamptic toxemia of pregnancy, although, individually, some of the nontoxemic patients had high values while some of the women with toxemia showed no elevations in the salt-retaining steroid.

More recently, D. Kumar, L. A. W. Feltham and A. G. Gornall (*Lancet* **I,** *541 (1959)*) studied the relationship of aldosterone and serum electrolytes in both normal pregnant females and those having pre-eclampsia. Maintaining their patients on a metabolic ward where it was possible to obtain known sodium intakes, they studied 17 normal pregnant women during the last trimester of their pregnancy. For 12 days the women were given a low salt diet which offered 1 g. (about 20 mEq) of sodium chloride per day. However for a six-day period, either at the beginning or end of the 12 days, sufficient salt was added to their diet to increase the intake to 8 g. (approximately 140 mEq) per day. At the end of this six-day period, a biopsy specimen of the rectus abdominis muscle was obtained under local anesthesia for use in chemical analysis.

In addition, seven patients with the signs and symptoms of pre-eclampsia were similarly studied, while three nonpregnant women, maintained on a normal salt intake, acted as controls.

Normal nonpregnant women in the childbearing age were found to excrete 4 to 11 micrograms of aldosterone per day when maintained on an 8 g. salt intake. However, when six pregnant females were maintained on both the normal salt intake (8 g.) and a low salt intake (1 g.), it was noted that on the normal salt intake they excreted an

average of 49 micrograms of aldosterone per 24 hours, while on the low salt intake they excreted 101 micrograms. Of the patients studied who were maintained on both salt intakes, only one individual on the normal salt intake had urinary aldosterone excretion greater than those on the low salt diet.

A determination of aldosterone was carried out on ten additional normal pregnant women while they were on either a low or normal sodium intake, and in general the aldosterone excretion agreed with the duplicate studies carried out on patients on both salt intakes.

In four patients showing mild pre-eclampsia, aldosterone levels in the urine tended toward lower values. Two patients who had recovered during the stabilization period had aldosterone levels in the urine which came closer to the average for normal pregnant women than the previous group.

The water, sodium, chloride and potassium contents of muscle biopsies were determined for the normal nonpregnant female and for the pregnant women who did or did not have pre-eclampsia. If the sodium, potassium, chloride and water contents of the biopsy were expressed per kilogram of muscle, there was no significant difference between the various groups.

However, from these measurements it was possible to calculate the value for the intracellular sodium content, and here differences were noted between the various groups. For the normal nonpregnant female maintained on a 8 g. salt intake, the intracellular sodium level was 8 to 9 mEq per liter of cell water. For normal pregnant women maintained on the same salt intake, the intracellular sodium ranged from 2 to 9 mEq per liter of water with one individual showing a value of zero. Four of the six values were below 4.8 mEq per liter. This was definitely below the levels found in the normal nonpregnant control subjects. Moreover, with the lower values of intracellular sodium concentration, the aldosterone excretion for the normal pregnant female was generally higher than the average of 46 micrograms per day.

In the case of five normal pregnant women who were maintained on a low sodium diet, the intracellular sodium varied from zero to 4.8 mEq per liter. Five of the six values were below 2.3 with two of these being zero. The average aldosterone excretion for the women in this group was 99 micrograms per day, a value which was considerably higher than that found in the pregnant females maintained on 8 g. of sodium chloride per day. There were no studies of the muscle intracellular sodium levels or aldosterone excretion in nonpregnant females maintained on the low sodium diet.

Two pre-eclamptic patients, who were given 8 g. of salt per day in their diet and recovered during the stabilization period, were found to have 5.2 and 1.5 mEq of sodium per liter of intracellular water, respectively. In a series of four patients, in whom the signs and symptoms of pre-eclampsia were still present while being maintained on a normal salt intake (8 g.), the average intracellular sodium concentration was 18 mEq per liter, a value which was considerably higher than that of any of the other groups studied. At the same time, the excretion of aldosterone was, on the whole, below those of any of the other pregnant patients.

The variation between individuals in regard to aldosterone excretion and intracellular sodium concentration was considerable in the various groups studied, possibly because of the variation caused by pregnancy. However, no significant measurement of the various means is given. The changes in intracellular potassium were not striking for any of the groups.

These investigators show clearly that there is increase of aldosterone in the urine during pregnancy, and that this increase by the third trimester may, on the average, be more than six-fold over the nonpregnant level when both groups are on a normal salt intake. On restricting salt intake, the level

of aldosterone in the urine may increase further in the pregnant patients. These investigators suggest that the increased excretion of aldosterone may be due in part to increased rate of production. They also believe that the aldosterone is probably derived from the maternal adrenals and not from the placental or fetal adrenals. The increase in aldosterone in normal pregnancy apparently is not caused by salt restriction per se. Moreover, since there is evidence that an increase in the plasma and extracellular fluid volume occurs during pregnancy, it is believed that the increase in aldosterone excretion is not due to a stimulation of a volume receptor mechanism responding to a decreased blood or plasma volume.

These preliminary studies indicate that in normal pregnancy there is usually a high aldosterone excretion accompanied by a tendency toward a low intracellular sodium level, while in pre-eclampsia lower aldosterone levels are accompanied by a tendency toward a higher level of intracellular sodium. Nevertheless, despite the changes noted between the pre-eclamptic patients and those with a normal pregnancy, care should be taken at this time in interpreting changes in the urinary aldosterone content and the intracellular sodium concentration with respect to their role in the pre-eclamptic toxemia. The possibility exists that they are the result of the toxemia rather than the cause. At the present time, a cause and effect relationship cannot be definitely established between these changes and the toxemia of pregnancy.

DIETARY FAT AND ENERGY AND PROTEIN METABOLISM IN MAN

Comparisons of low and high-fat diets, isocaloric and isonitrogenous, revealed no differences in the energy expenditures and nitrogen balances in two pairs of adult males studied in a respiration calorimeter.

Extensive studies in albino rats have shown that high-fat diets are more efficiently utilized than isocaloric and isonitrogenous low-fat diets (*Nutrition Reviews* **5,** *100* (*1947*); A. Black, C. E. French and R. W. Swift, *J. Nutrition* **37,** *275* (*1949*); Black *et al.*, *Ibid.* **37,** *289* (*1949*); Swift and Black, *J. Am. Oil Chem. Soc.* **26,** *171* (*1949*)). They have also shown that increasing quantities of dietary fat result in decreasing heat production in both growing and mature rats (*Ibid.*) and that increasing quantities of fat in isocaloric, restricted protein diets are associated with superior energy utilization, increased body gains of fat and energy and decreased heat production (French, Black and Swift, *J. Nutrition* **35,** *83* (*1948*)).

To determine whether these findings also apply to humans, Swift *et al.* (*J. Nutrition* **68,** *281* (*1959*)) have studied the energy and nitrogen metabolism of two pairs of well motivated young men who were 23 years of age, 154 pounds in weight, with normal basal metabolisms. These young men were fed low- and high-fat isocaloric and isonitrogenous diets which provided 3035 calories and 97.5 g. of protein daily, and contained respectively 4.86 per cent and 34.50 per cent fat on a dry weight basis and 9.8 per cent and 60.1 per cent fat on an energy basis. They included other nutrients in excess of National Research Council Allowances and both were composed of the same main food items. The low- and high-fat diets were served in separate dining rooms to minimize any untoward reactions between the subjects.

Energy metabolism was determined for each pair of subjects on both dietary regimens during four 24 hour periods in the respiration calorimeter. The first period took place three days after assignment to a particular diet, while the second was ten days after assignment. Both subjects of a

pair were in the respiration calorimeter together during the 24 hour measurement of heat (including water vaporization) and carbon dioxide production, and a limited amount of prescribed exercise was performed by them at this time. Between the two calorimeter periods, nitrogen balances were determined for each subject on a given diet during a five day collection of feces and urine. One pair of subjects received the low-fat diet first with subsequent assignment to the high-fat diet. The reverse order was followed with the other pair. Details of the studies were similar to those of previous investigations recently described (Swift *et al.*, *J. Nutrition* **65,** *89 (1958)*).

One pair of subjects lost one pound and the other pair one-half pound during the course of the studies. Heat production figures revealed caloric gains of over 1000 calories per pair of subjects per 24 hours. The average daily heat production from the low-fat diet for each pair was 4990 calories and, from the high fat diet, 4993 calories. One pair of subjects produced 3.2 per cent more heat on the low-fat than on the high-fat diet, but the other pair showed a difference of 3.3 per cent in the opposite direction. Carbon dioxide production was definitely less on the high fat diet.

Average daily nitrogen balances for each pair were +0.27 g. and +3.21 g. on the low-fat and +1.91 and +0.42 g. on the high-fat diet. The percentage of total heat eliminated by the vaporization of water averaged 30.2 per cent for all periods and, in the authors' opinion, was not affected appreciably by the composition of the diets.

Previous studies (Swift *et al.*, *J. Nutrition* **65,** *89 (1958)*) have shown that the 24 hour energy metabolism remained the same for seven weeks of a given dietary regimen. In this study, however, both pairs of subjects, while consuming the low-fat diet, produced about 3 per cent more heat and carbon dioxide on the tenth than on the third day. The authors suggest, especially in the light of the work on albino rats, that longer periods on the low-fat regimen might reveal consistently higher heat losses. However, this would not be associated with differences in metabolizable energy, but rather with the division of metabolizable energy into heat production and body gain. In the human studies, the differences between low- and high-fat diets in terms of digestible energy and metabolizable energy were less than one per cent.

Despite the possibility that more prolonged studies might have resulted in different interpretations, the authors conclude that there is no appreciable difference in the energy utilization by humans of isocaloric and isonitrogenous diets containing widely different amounts of fat. More prolonged studies to evaluate the importance of adaptation and to explain the large caloric gains and positive nitrogen balances without accompanying weight gains would be of considerable interest.

EGG PROTEIN REQUIREMENT OF YOUNG ADULTS

The requirement for nitrogen balance of the essential amino acids of whole egg protein has been determined in young men and women at two levels of nitrogen intake.

In a series of nitrogen balance studies in young men and women students, M. E. Swendseid *et al.* (*J. Nutrition* **68,** *203 (1959)*) have attempted, first, to evaluate egg protein solely as a source of essential amino acids in the presence of adequate total nitrogen intake and, second, to obtain evidence as to whether essential amino acid requirements for nitrogen equilibrium are influenced by the total amount of nitrogen in the diet.

Nitrogen balances were measured in five men and six women fed varying amounts of whole egg in successive dietary periods, with total nitrogen intakes of 6.5 or 13 g. per day

held constant by the addition of a mixture of glycine and diammonium citrate. Two men and four women were studied at both levels of nitrogen intake.

All subjects first consumed a diet of ordinary foods containing 6.5 g. nitrogen for seven days, or until nitrogen equilibrium was established. They then ingested an isonitrogenous, semisynthetic diet composed of low-protein fruits and vegetables, centrifuged butter, sucrose, cornstarch, minerals and vitamins with essential amino acids provided as hard-cooked, sieved whole eggs. This diet gave them from 45 to 50 calories per kg. of body weight per day. The whole egg fed per day was distributed over three meals and varied, in successive dietary periods of seven or eight days each, from 65 to 120 g. daily on the 6.5 g. per day total nitrogen intake, and from 80 to 200 g. daily on the 13 g. per day total nitrogen intake. Nitrogen analyses were performed on food samples, daily urine collections and five or ten day pools of feces.

On the 6.5 g. total nitrogen intake, only one subject (male) stored nitrogen (+0.30 g. per day) on 80 g. whole egg per day. Only one subject (another male) failed to store nitrogen (−0.49 g. per day) on 100 g. whole egg per day. By W. C. Rose's criteria of adequacy (*Fed. Proc.* **8,** *546 (1949)*; *Nutrition Reviews* **8,** *118 (1950)*) for nitrogen equilibrium or nitrogen storage, five of seven subjects received adequate amounts of essential amino acids from 100 g. of whole egg. By the "zone of equilibrium" criteria of R. M. Leverton *et al.* (*J. Nutrition* **58,** *59 (1956)*; *Nutrition Reviews* **14,** *269 (1956)*), five of seven subjects received adequate amounts of essential amino acids from 80 g. of whole egg. By either set of criteria, none received adequate amounts from 65 g. of whole egg.

Since the egg protein requirement of these seven subjects ranged from 10 to 15 g. per day and since E. E. Sumner and J. R. Murlin (*J. Nutrition* **16,** *141 (1938)*) found a requirement of approximately 20 g. when they fed egg as the main source of dietary nitrogen, the present authors conclude that, when egg is the sole protein source, the total nitrogen content rather than an essential amino acid becomes the limiting factor in nitrogen balance experiments.

In this study the observed range of egg protein requirement expressed as essential amino acid nitrogen (0.6 to 0.9 g. per day) lies above values used in two other investigations where essential amino acid mixtures were fed in ratios based on their individual requirements. Rose and R. L. Wixom (*J. Biol. Chem.* **217,** *997 (1955)*) fed young men diets containing 1.42 g. essential amino acid nitrogen daily, which was twice the minimum requirements (*i.e.*, safe intakes) of the eight essential amino acids. They were able to maintain nitrogen equilibrium on diets containing from 10.0 down to 3.5 g. total nitrogen per day. On the other hand, Swendseid and M. S. Dunn (*J. Nutrition* **58,** *507 (1956)*) maintained young women in nitrogen equilibrium on an equivalent of 0.46 g. nitrogen given as essential amino acids. The quantitative differences noted emphasize the inadequacy of present knowledge concerning the efficiency of different amino acid patterns and the availability of amino acids in food proteins.

The present authors have attempted also to identify methionine as the limiting amino acid in egg protein by adding 300 mg. of that amino acid to the 80 g. whole egg diet. However, in the three subjects studied, nitrogen loss was reduced but not abolished in two and remained unchanged in a third.

On the 13 g. total nitrogen intake, three of six subjects stored nitrogen on 80 g. whole egg per day; six of seven, on 100 g. per day; two of six, on 120 g. per day; and four of six, on 150 g. per day. One subject approached but did not reach equilibrium on 200 g. per day. Viewed in another fashion, three subjects were in equilibrium or positive balance on 80 g. per day; three changed from negative balance to positive balance on receiving

100 g. per day; and two changed from negative to positive balance on going from 120 to 150 g. per day. There was greater variation in the requirement for egg as a source of essential amino acids at the 13 g. than at the 6.5 g. total nitrogen level. Interpretation is also made difficult by the fact that not all subjects participated in all phases of the investigation.

Of the six subjects studied on both total nitrogen intakes, two had increased nitrogen requirements at the higher level, one had a slightly decreased requirement and three had essentially the same requirement. These studies failed, therefore, to demonstrate a consistent relationship between amino acid requirements and nitrogen intake. The authors suggest that these results may be based on individual differences in the utilization of nonessential nitrogen at the two intake levels. They also point out that a similar study in old men (S. G. Tuttle *et al.*, *Metabolism* **8,** *61 (1959)*) demonstrated an increase in essential amino acid requirements with increasing nitrogen intake.

The need for more studies utilizing mixtures of pure L-amino acids and employing more effective means for evaluating amino acid availability in food proteins, is again emphasized by the findings of these experiments.

SERUM AND TISSUE VITAMIN B_{12} CONCENTRATIONS IN MAN

Measurements of serum and tissue vitamin B_{12} concentrations in a large number of normal subjects of various ages and in chronically ill patients have revealed no evidences of subclinical vitamin B_{12} deficiencies.

The reported successes of large and frequently administered doses of vitamin B_{12} in the treatment of a wide variety of clinical conditions (trigeminal neuralgia, herpes zoster, subnormal growth in children, hepatitis, certain skin conditions, certain neuropathies other than combined system disease, osteoporosis, neurofibromata) have led J. A. Halsted, J. Carroll and S. Rubert (*New Engl. J. Med.* **260,** *575 (1959)*) to postulate three reasons for these clinical benefits. First of all, they suggested, the patients may have subclinical deficiencies not manifest by the usual picture of Addisonian anemia. Secondly, the vitamin may have a specific, though yet unknown, pharmacologic action unrelated to nutrition; and, finally, the patient may merely respond to the vitamin as to a placebo.

The authors undertook to investigate the first postulate by obtaining data on the serum and tissue concentrations of vitamin B_{12} in a wide variety of pathologic states in an effort to determine whether a vitamin B_{12} deficiency might be shown to exist in other than the five principal conditions of clinical B_{12} deficiency (inadequate dietary B_{12}, deficient intrinsic factor, intestinal malabsorption, "blind loop" syndrome and competition for B_{12} by *Diphyllobothrium latum*).

Microbiological assays for vitamin B_{12} were performed using *Lactobacillus leichmannii* 4797 according to the method of H. R. Skeggs *et al.* (*J. Biol. Chem.* **184,** *211 (1950)*), turbidometric readings being made after 72 hours incubation. The method gave recovery values averaging 90, 101 and 92 per cent when 80, 200 and 800 $\mu\mu$g. per ml. were added to four replicates of pooled human serum, while individual recoveries ranged from 58 to 115 per cent. When the three sera were each assayed eight times, the greatest variation was 8.5 per cent from the mean. Six assays at weekly intervals in each of ten normal subjects revealed that the greatest variation was 7.0 per cent from the mean.

During the study, serum was assayed in 632 persons, 333 of which were controls and

the remainder falling into one of 16 categories of chronic disease. Data were analyzed by Student's "t" test.

The mean serum vitamin B_{12} concentration for the entire control group was 470 ± 196 μμg. (standard deviation) per ml. There was no recognizable decrease in mean serum B_{12} concentration with age until the tenth decade, and the decrease in the tenth decade gave a probability of less than 0.10 (See *Nutrition Reviews* **14,** *294 (1956)*). No significant sex differences were noted.

Sixteen patients with pernicious anemia in relapse had a mean concentration of 37 μμg. per ml. and a range of 16 to 104. Four alcoholics with inadequate intakes of B_{12} had a mean of 104 μμg. per ml. and a range of 28 to 140.

Other types of patients were reported to have the following mean concentrations and ranges: 12 with tropical sprue, 89 (31 to 150); 15 with duodenal ulcer, 593 (277 to 1155) (p less than .02 when compared to normals); ten with subtotal gastrectomy, 439 (277 to 760); 13 with nonmegaloblastic anemia 575 (125 to 1371); 15 with widespread carcinoma, 499 (140 to 1250); 25 with degenerative neurologic disease, 491 (142 to 1140); 60 with liver disease, 728 (150 to 3550) (p less than .001 when compared to normals); 41 diabetics with retinopathy (renal disease excluded), 502 (230 to 884); eight diabetics with retinopathy and severe renal disease, 800 (345 to 1105) (p less than .01 when compared to normals; 24 diabetics without retinopathy or renal disease, 533 (178 to 926); six with chronic myelocytic leukemia, 2150 (560 to 4600); four with nonmyelocytic leukemias, 571 (354 to 802); and 40 with miscellaneous chronic disease, 471 (175 to 1652).

Assays for vitamin B_{12} concentrations were performed on normal and cirrhotic liver, kidney, spleen, peripheral nerve, brain, bone marrow, intestinal mucosa and carcinoma. Eighty-seven normal livers had a mean and standard error of 614 ± 22 μμg. per g. of wet tissue (See *Nutrition Reviews* **15,** *192 (1957)*). Fourteen cirrhotic livers contained 263 ± 210 μμg. per g. of wet tissue. The difference was highly significant. Total liver B_{12} content in cirrhotics was roughly half that in normals. Except for kidneys, which had approximately one-sixth the B_{12} concentration of normal livers, all tissues assayed had relatively small B_{12} concentrations (14 to 41 μμg. per g. of wet tissue). Neither bone marrow nor intestinal mucosa, where cell division is most rapid, contained more vitamin B_{12} than brain or nerve, where cell division is slowest.

The authors conclude that their data fail to substantiate the postulate that subclinical deficiencies of B_{12} may occur in various chronic diseases and in old age. They suggest that the reported beneficial effects of vitamin B_{12} in cases where deficiency does not exist are probably the result of suggestion by placebo.

However, the basic hypothesis on which their study is founded (*i. e.*, that serum and tissue concentrations of vitamin B_{12} would reflect the presence or absence of a minor deficiency state), though grounded in tradition, may prove to be untenable in the specific case of vitamin B_{12}. Ample evidence exists that serum B_{12} concentrations are a function of qualitative and probably quantitative differences in the B_{12}-binding proteins (R. S. Mendelsohn *et al.*, *Blood* **13,** *740 (1958)*). Among the general population on average mixed diets, these concentrations do not correlate with the amount of B_{12} absorbed (I. B. Weinstein and D. M. Watkin, *Proc. Am. Assoc. Cancer Res.* **2,** *355 (1958)*). It should be noted that B_{12} exists in plasma in a loosely bound ("free") state and in a tightly bound state (I. B. Weinstein, S. M. Weissman and D. M. Watkin, *Fed. Proc.* **18,** *551 (1959)*). The tightly bound B_{12} will not support growth of *L. leichmannii* and, to be assayed, must first be liberated by heating in an extraction solution. In other organs, B_{12} exists in a tightly bound form.

It is possible, therefore, that the concentration of tissue B_{12} may represent the

quantity in storage and thus have little bearing on possible disorders in biochemical systems responsible for liberation and utilization of the vitamin. Future work concerning the role of B_{12} in non-overt deficiency states might well include the investigation of B_{12} kenetics, further characterization and quantification of B_{12}-binding proteins in serum and tissues, as well as studies with metabolic antagonists of vitamin B_{12}.

THE DIGESTION OF CARBOHYDRATES BY THE BABY PIG

Newborn piglets use starch and sucrose inefficiently because of low levels of carbohydrases other than lactase in their intestinal tracts. The levels increase rapidly after the first week.

Under natural conditions the only carbohydrate ingested by a young suckling animal is lactose. However, it is economically advantageous to stimulate faster growth in the baby pig by providing food in addition to the sow's milk, sometimes eliminating the sow's lactation period altogether. Since other carbohydrates are cheaper to provide than lactose there is practical interest in the question as to whether or not they are equally useful as sources of digestible energy for the piglet.

It has been the general conclusion of investigators, as reviewed by T. R. Preston (*Proc. Nutr. Soc.* **17,** *63 (1958)*), that the newborn pig is able to use only lactose and glucose, but that within a few days it is able to utilize other carbohydrates to some extent, and that well before the end of the normal suckling period the piglet can dispense with lactose as its staple source of energy. These conclusions have been based on both the presence of specific enzymes in the intestinal tract (C. B. Bailey, W. D. Kitts and A. J. Wood, *Canad. J. Agr. Sci.* **36,** *51 (1956)*; D. B. Hudman *et al., J. Agr. Food Chem.* **5,** *691 (1957)*), and on the response of reducing sugars in the blood of fasted piglets to the administration of different carbohydrates (A. M. Dollar, K. G. Mitchell and J. W. G. Porter, *Proc. Nutr. Soc.* **16,** *xii (1957)*; *Ibid.* **17,** *xv (1958)*).

A recent British study by D. M. Walker (*J. Agr. Sci.* **52,** *352, 357 (1959)*) provides additional information on this subject. Altogether 40 baby pigs were slaughtered at intervals from birth to five weeks of age, and their stomach, intestines and pancreas removed immediately and preserved at a low temperature. The pH of the stomach, duodenal and small intestine contents showed no trends with age and were usually in the ranges 3.5 to 4.0, 5.5 to 7.0 and 5.8 to 6.5, respectively. In contrast to findings with older pigs, none of these young showed free hydrochloric acid in their stomach contents. Analysis for total chloride (as salt) in the stomach showed less than 4 mg. per g. of wet contents in the first two weeks, and 4 to 7 mg. per g. thereafter.

Enzyme extracts were prepared from finely ground pancreatic tissue, and also from a mixture of duodenal and small intestinal tissue. The extracts were tested for amylotic activity towards sodium starch glycollate in phosphate buffer of pH 8.5. This pH was chosen because the extracts then appeared more stable than at pH 7.0, which was the point of optimum enzyme activity. The effect of the enzyme was measured by the change in blue coloration with added iodine after a fixed period.

The pancreatic amylase activity was expressed in arbitrary units. When the total pancreatic activity was calculated per kg. of total body weight, the mean value for pigs killed in the first week of life was only 5 per cent of that for four- to five-week-old animals, but in the second week of life the value rose to about 60 per cent.

For all ages, the absolute activity of the small intestine as a source of amylase was considerably less than that of the pancreas when determined in the same way. The

activity at five weeks of age was also only about three times that of the newborn animal. Most of the increase again occurred in the second week of life.

The small intestine extracts were also assayed for lactase, sucrase and maltase activity at the pH optima of 6.0, 6.5 and 7.0, respectively. This was done by adding the particular substrate, and measuring the extra reducing activity present after one hour's digestion at 39° C. By calculating back to the total activity of the small intestinal tissue (+ duodenum) in each carcass, and then dividing by total body weight, the results were expressed in units of mg. disaccharide hydrolysed per hour per kg. body weight.

In the case of lactase, the seven pigs killed in the first week of life showed 3000 to 7000 units of activity. Corresponding figures in the second week were 750 to 3300, and in the fifth week the values obtained were all below 1200. However, this did not indicate an absolute decline with age in the power of the pig to hydrolyse lactose. If the activity is calculated per pig, it remains relatively constant at 6000 to 7000 in the first five weeks of life.

In this experiment the pigs were all nourished entirely from sow's milk so that there was no pressure to adapt to other carbohydrates, a possibility if they had been receiving a supplement containing other carbohydrates.

Whether the lactase activity should be considered as constant or declining with age, *i.e.*, whether it is more meaningful to express the activity per pig or per kg. body weight, is a question which should perhaps be studied with relation to food intake per pig. Presumably the lactase activity will increase to some extent. However, there is no reason to believe that the enzymes are inadequate at any point, so that we may conclude merely that the margins of safety are particularly large in the first period.

In the case of both sucrase and maltase there was a rapid increase in enzyme activity from the first to the fifth week, regardless of the method of calculation. Expressed per kg. body weight, it rises from approximately 70 to 1500 mg. for sucrase, and from approximately 300 to 2000 mg. for maltase. Expressed per pig, the differences are of course much greater; from 60 to 12000 for sucrase and from 270 to 22000 for maltase.

Walker suggests that the factor limiting the utilisation of starch by the one-week-old pig is not the level of amylase activity, but the limited ability to complete the hydrolysis of the maltose resulting from the activity of these enzymes. However, H. M. Cunningham and G. J. Brisson (*J. Animal Sci.* **16,** *574 (1957)*) came to the opposite conclusion from their own studies, which were conducted on generally similar lines.

As the authors themselves point out, calculations of this sort are on a precarious basis when they derive from the enzyme activities of the whole tissues, and we have no knowledge of their natural rate of release into the intestinal tract. We know also that pH and other variables can influence their activity. Nevertheless, these experiments are of value in unravelling a practical feeding problem and in providing a rational basis for the choice of milk substitutes and the age at which they can usefully begin to be fed.

LONG-TERM STUDIES OF ANTIBIOTIC GROWTH STIMULATION

Although it is stated that the addition of antibiotics to poultry feeds no longer produces a growth response, there are some laboratories where the response appears to have been maintained over the years.

There has been a number of unpublished statements implying that the growth-stimulating effects of antibiotics added to feeds are not as great as they were a number of

years ago. A commercial bulletin (*Research and Development Status Report on Oleandomycin, No. 2*, Pfizer, 1957) indicates that "... there has been a gradual increase in the growth rate of control birds with a consequent lessening of the spread in weight between controls and those fed antibiotics."

A number of years ago, D. A. Libby and P. J. Schaible (*Science* **121,** *733* (*1955*)) reported work which confirmed this claim. They raised newly hatched chicks on commercial rations, giving half of each group an antibiotic and the other half the same ration without the supplement.

All chicks were raised in the old facilities in order to enhance any effect the antibiotic might have. According to the work of M. E. Coates and co-workers (*Nutrition Reviews* **10,** *270* (*1952*); **12,** *13* (*1954*)), chicks reared in the old environment show a marked growth response to antibiotics while such an effect is not seen when they are reared in new quarters or in a clean environment. Moreover, in the clean environment, the growth of the chicks on the control or unsupplemented diet is as good as, or better than, that of the birds in the old environment receiving the antibiotic-supplemented feed.

During the first year, Libby and Schaible (*loc. cit.*) found that in 75 experiments the antibiotic-supplemented birds gained an average 19.0 per cent more than their unsupplemented controls. However, the difference between the two groups gradually decreased from 1950 through 1953 when it amounted to 3.3 per cent. Over the same period, the control birds gradually increased in weight when compared with their 1950 counterparts, so that by the end of 1953 the percentage increase had risen to 19.1. Thus the disappearance of the antibiotic growth stimulating effect was attributed to the improved growth of the controls.

The per cent mortality of the birds at six weeks of age gradually decreased from 8.5 to 2.8 in the last year of the study. This reduced mortality rate was associated with a reduction in the number of chicks that had to be culled and the individual variability in growth rates.

Libby and Schaible suggested that a reduction in the "germ load or disease potential" had occurred in their laboratory as a result of the long-term use of antibiotics.

In contrast to the above work, however, are two reports from England. Coates and M. K. Davies have reported that birds raised under simulated commercial conditions continue to show an increased growth response even to the fourth generation when antibiotics are added to the diet (*Brit. J. Nutrition* **13,** *205* (*1959*)).

In 1955 they divided about 200 unsexed chicks into two groups. One group received procaine penicillin in the diet at a level of 45.5 g. per ton of chick mash and, starting at eight weeks of age, 10 g. per ton of breeder's or grower's mash. The other group was maintained on the same diet without the antibiotic. The investigators used levels of antibiotics which were higher than those used commercially to allow for possible losses during storage.

The studies were carried out in quarters that had been used for rearing chicks over a number of years. Moreover, to accentuate the condition, the tests were made while the chick rooms were occupied to capacity.

When fertile eggs were secured, an unselected sample of 90 to 250 eggs from each group was incubated. On hatching, half of the chicks from each group was placed on the supplemented diet, while the other half was kept on the basal diet. In each succeeding generation, eggs were incubated only from the birds which had been placed on the same diet as their parents.

The growth response to penicillin was measured by the weight gains of the chicks during the first four weeks. In each of the four generations, the supplemented chicks gained approximately 8.5 per cent more weight during the first four weeks of life than did their controls. However, there was no change from generation to generation in

the magnitude of the growth response secured with penicillin.

The penicillin-supplemented ration produced the same growth response in offspring of unsupplemented parental birds as it produced in offspring where the parental birds had received the supplemented ration. The mortality of the chicks (2 per cent) was not affected by the presence of penicillin in the diet.

A flock of chicks raised under commercial broiler conditions was studied in a similar way by Coates and Davies (*loc. cit.*). As in the above experiment, weight increase was measured during the first four weeks of the chicks' life. From 1952 to 1957 the growth increase of the chicks receiving penicillin was approximately 11 per cent greater than that of the controls. Egg production was recorded in a small group of hens not subjected to normal commercial culling and no essential difference was found in the number of eggs produced by the groups receiving feed either with or without penicillin.

A similar experiment was carried on by R. C. Jennings (*World's Poultry Science J.* **13,** *284 (1957)*). A chick mash containing 4 g. of penicillin per ton was fed until the chicks were eight weeks of age. The control group received the same ration without the penicillin. No antibiotic was fed after the eighth week. The offspring secured when the chickens in the control and supplemented groups were crossed were put through the same regimen.

In the first two years of the study, the penicillin-supplemented chicks gained 8 per cent more weight through the eighth week of life than the controls, although in the third year the antibiotic did not affect growth. By the fourth year, the penicillin-supplemented birds were 4 per cent heavier.

Again, the growth-promoting response elicited by the addition of small amounts of penicillin to a commercial chick feed appears to have been maintained over a period of years. Moreover there is no indication from these studies that the antibiotic had any adverse effect attributable to the development of resistant organisms.

The above observations suggest that the continued use of antibiotics in the same laboratory does not necessarily lead to a marked increase in harmful bacteria grown resistant to the antibiotic. There is also no indication that the antibiotics have any cumulative adverse effect on growth, egg production or hatchability. Furthermore, these studies suggest that there may be as much variation from year to year in the growth response elicited by antibiotics within the same laboratory as there is among the various laboratories engaged in the same work.

The elusiveness of the antibiotic growth response in many laboratories and the complexity of the factors involved are largely responsible for the absence of an explanation as to how the antibiotics act. For this reason, it is difficult to determine whether the variations in antibiotic growth response are due to changes in the birds or to the environment.

EFFECT OF ANTIOXIDANTS ON VITAMIN E DEFICIENCY IN CHICKS

Although two synthetic antioxidants can prevent some symptoms of vitamin E deficiency in chicks, certain differences indicate that the action of the vitamin E is not simply as an antioxidant.

The symptoms of vitamin E deficiency in chicks include muscular degeneration, exudative diathesis and encephalomalacia (*Nutrition Reviews* **10,** *149 (1952)*; **14,** *141 (1956)*). Classically, these symptoms have been produced by placing the chicks on a diet as low

in vitamin E as possible and feeding some highly unsaturated fat, such as fish oil. As an aid in depleting the vitamin E of the chick, the hen may be on an E-deficient diet. Prevention and cure of this deficiency is accomplished by inclusion in the diet of small amounts of tocopherols or (usually) larger amounts of certain antioxidants such as methylene blue, N, N^1-diphenyl-paraphenylenediamine (DPPD), 2,6-ditertiarybutyl-4-methoxyphenol (BHA) 2,6-ditertiarybutyl-4-methylphenol (BHT) and others.

These results have led to the question of the specificity of the tocopherols as vitamins E. Do the synthetic antioxidants replace tocopherol in its metabolic functions or do they simply protect it from oxidative destruction (*Nutrition Reviews, loc. cit.*)?

A further attempt to answer this question has been made by L. J. Machlin, R. S. Gordon and K. H. Meisky (*J. Nutrition* **67,** *333* (*1959*)). Three purified vitamin E-deficient diets were fed to week-old chicks for three weeks. These differed mainly in content of protein, fat (stripped lard) and carbohydrate (glucose to make up the weight). The low casein, low fat diet contained 15 per cent casein, 10 per cent gelatin, and 4 per cent lard. The high casein, high fat diet contained 20 per cent casein, 15 per cent gelatin, and 16 per cent lard; and the assay protein (a soybean protein) diet contained 35 per cent assay protein and 16 per cent lard. The low casein diet had approximately 0.09 I.U. of vitamin E per pound, while the other two had about four times this amount.

Chicks on the low casein, low fat diet exhibited muscular degeneration and a high mortality, evidently due to destruction of vitamin A (which dropped to very low levels in these birds). Three antioxidants, BHT, 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline and calcium DL-2-hydroxy-4-methyl thiobutyrate at 0.1, 0.01 and 0.6 per cent, respectively, served to prevent both vitamin A destruction and death and alleviated the muscular degeneration to some extent. Surprisingly, lauroyl peroxide, which might be considered a pro-oxidant, also had some beneficial effect on the death rate. It is unlikely, however, that any of this peroxide was absorbed as such, and some indirect action may have been responsible for this slight effect. With the diets containing 16 per cent lard, encephalomalacia or all three symptoms resulted.

With the high casein diet supplemented with 0.6 per cent arginine, the thiobutyrate derivative (at 0.6 per cent) did not prevent encephalomalacia, while the quinoline derivative (at 0.02 per cent) prevented this disease but not the muscular degeneration. On the other hand, 0.004 per cent DL, alpha-tocopherol prevented both symptoms as well as the liver peroxide accumulation, indicated by the thiobarbiturate test (M. L. C. Bernheim, A. Ottolenghi and F. Bernheim, *Biochim. Biophys. Acta*, **23,** *431* (*1957*)), which was not prevented by the two synthetic antioxidants.

In contrast to the greater *in vivo* efficiency of tocopherol, it was found in *in vitro* experiments that the quinoline antioxidant was more than eight times as effective as tocopherol in preventing peroxide formation in liver homogenates of chicks on the assay protein diet. This antioxidant was also very effective in preventing oxidative rancidity in the diet.

It seems evident from this study, as from previous experiments (*Nutrition Reviews* **17,** *174* (*1959*)) that tocopherol functions as an antioxidant in the living tissues as well as in protection of dietary constituents. Its prevention of peroxide formation in liver homogenates of chicks on an E-deficient diet as well as its known protection of liver vitamin A attest to this fact. Moreover, these functions appear to be fairly non-specific since the synthetic antioxidants can replace them. However, it is significant that the quinoline derivative was more effective *in*

vitro while alpha-tocopherol was more so *in vivo*.

How many of the other symptoms of vitamin E deficiency may be due to its antioxidant properties cannot be said with certainty from the results of the present experiments. However, several clues are pointed out by the authors. In the low casein diet, the vitamin E content was so low (0.09 I.U. per pound) that if the effective antioxidants act merely by protecting vitamin E, the requirements of the latter must be extremely small. That this may indeed be the case was indicated by the experiments of Dam and his co-workers, who found that if the vitamin E were completely removed from the diet of rats, methylene blue no longer prevented certain deficiency symptoms (*Nutrition Reviews* **15,** *116* (*1957*)). However, although peroxide formation in the livers of deficient birds appeared to be correlated with muscular degeneration, no such correlation could be found for the case of encephalomalacia. Moreover, it is difficult to explain why muscular degeneration appeared to be the sole symptom on the low casein (4 per cent lard) diet while encephalomalacia resulted as the major symptom on the high casein (16 per cent lard) diets.

Thus, these experiments have added evidence to the already large accumulation on the subject of the mechanism of action of vitamin E, but have not solved the problem. The solution may come from experiments on a somewhat different scale; the relationship of tocopherol, coenzyme Q and the terminal oxidation enzymes.

SEX HORMONES AND CHOLESTEROL METABOLISM

Estradiol treatment was found to stimulate endogenous cholesterol biosynthesis in castrated rats. This suggests that the higher serum cholesterol concentration of the female rat may be related to estrogenic activity.

Among the many factors known to influence the concentration of serum cholesterol in the rat are the sex hormones, estradiol and testosterone. The serum cholesterol concentration of the female rat is normally higher than that of the male and treatment with estradiol causes an elevation in the concentration of serum cholesterol and other lipid components in the male and the castrated rat (L. C. Fillios, *Endocrinology* **60,** *22* (*1957*); Fillios and S. B. Andrus, *Fed. Proc.* **16,** *356* (*1957*)). Treatment of female rats with testosterone lowers the concentrations of serum cholesterol and other serum lipid components (Fillios and G. V. Mann, *Circulation Res.* **4,** *406* (*1956*)).

These observations have been extended by Fillios, R. Kaplan, R. S. Martin and F. J. Stare (*Am. J. Physiol.* **193,** *47* (*1958*)) to provide information about the relationship between age and serum cholesterol concentration in both male and female rats, and about the relationship stage of the estrous cycle and serum cholesterol concentration in the female rat. They have also made an effort to determine why the serum cholesterol concentration is higher in the female.

The rats used in the study were fed ad libitum on a commercial laboratory chow. Blood for serum cholesterol determinations was collected from the tail vein, except in terminal studies in which blood was obtained by cardiac puncture.

The initial study consisted of a comparison of the serum cholesterol concentrations of male and female rats from birth until about 18 months of age. At birth, the average value for serum cholesterol concentration was 72 mg. per cent and there was no sex difference. During the nursing period, serum cholesterol concentrations rose to 86 mg. per cent for males and 93 mg. per cent for females at 21 days of age. The difference between these values was not significant, but

after eight weeks, when the value for males had dropped to 68 and that for females was 90 mg. per cent, there was a highly significant sex difference. Significant differences were evident thereafter until 540 days of age when the value for males was 65 and that for females was 78 mg. per cent.

Next, the authors investigated the effect of the stage of the estrous cycle on the serum cholesterol concentration of female rats four to six months of age. The stage of the estrous cycle was determined by examining vaginal smears and, in terminal experiments, by an examination of the uterus and ovaries. Blood samples for serum cholesterol determinations were taken from five to 12 rats at short intervals during the four and a half day estrous cycle.

When the values for serum cholesterol concentration were plotted against the stage of the estrous cycle, the curve obtained had a peak of about 95 mg. per cent. This occurred during the proestrous to estrous phase, the period of greatest activity of the ovarian follicles. Following estrous, the serum cholesterol concentration fell gradually until during the diestrous phase, the phase of low follicular activity, serum cholesterol concentrations of from 65 to 75 mg. per cent were obtained.

The final group of experiments were undertaken to study the influence of sex hormones on endogenous cholesterol synthesis. In these studies intact, ovariectomized and orchidectomized rats, six months of age, were used. Rats in each of the last two groups were given injections of 0.05 ml. of sesame oil, or an equal quantity of sesame oil containing either 0.02 mg. of estradiol dipropionate or 1 mg. of testosterone propionate three times a week for three weeks. They were then injected with 10 mg. (159 microcuries) of sodium acetate-1-C^{14}, and 24 hours later five to six rats from each group were bled and the C^{14} activity of the serum cholesterol was determined.

Similar measurements were made at 24 hour intervals for four days. Then, by plotting the values for the logarithm of specific activity of the isolated cholesterol against time and extrapolating the curve for each group to zero time, a relative measure of endogenous cholesterol biosynthesis was obtained.

The results of these estimations indicated that endogenous cholesterol biosynthesis was higher in intact female rats than in intact males. Treatment with estradiol increased the rate of endogenous cholesterol biosynthesis in both ovariectomized and and orchidectomized rats, whereas treatment with testosterone depressed endogenous cholesterol biosynthesis in both groups. The observations on the relative rates of cholesterol biosynthesis appeared to correlate well with the observed serum cholesterol concentrations for the various groups. In one experiment in which it was tested in orchidectomized rats, progesterone was found to be without effect.

These observations lead to the conclusion that endogenous cholesterol biosynthesis in the rat is stimulated by estradiol. Such an effect could account for the higher average cholesterol concentration in the serum of female rats and for the fact that the highest concentrations in the female occur during peak of follicular activity in the estrous cycle.

The authors caution that conclusions about the role of sex hormones in regulating serum cholesterol concentration in the human should not be made on the basis of these results. They cite conflicting reports concerning the influence of estrogens on hypercholesteremia in man and point out that human hypercholesteremia is frequently of unknown origin.

The experiments reported above were carried out on animals fed a stock diet, presumably low in cholesterol and possibly containing some plant sterols. In any case, the regimen resulted in generally low serum cholesterol levels. The question as to whether the effects of treatment with sex

hormones would have been so marked if the animals had been fed a diet causing hypercholesteremia is unanswered. Experiments performed under such conditions would probably parallel more closely the situation in the human.

ABSORPTION IN METHIONINE DEFICIENCY

Methionine deficiency does not seem to result in impaired digestion or absorption from the intestinal tracts of chicks.

Although it has often been demonstrated that deficiencies of amino acids will cause decreases in the activities of various enzymes, little or no information is available on the effect of an amino acid deficiency on protein digestion and absorption in the intestinal tract.

J. J. Pisano, C. M. Paine and M. W. Taylor (*J. Nutrition* **67,** *213 (1959)*) have examined the question in terms of digestibility studies on white leghorn chicks. Samples of the contents from various levels of the gastrointestinal tract were tested (instead of the feces) so that it was possible to follow digestion and absorption of protein nitrogen as food passed down the tract. This approach gave a basis for comparison and also yielded new information on little studied processes of absorption and digestion in the chick.

Digestion estimations were made by the chromic oxide indicator method described by A. F. Schürch, L. E. Lloyd and E. W. Crampton (*J. Nutrition* **41,** *629 (1950)*). The method involves the incorporation of a known amount of a completely indigestible, non-absorbable and inert material, chromic oxide, in the food. A coefficient of digestion or absorption for the nitrogen of the food can be calculated from the ratios of chromic oxide and nitrogen in the food and in the contents of the tract.

One group of ten chicks was fed on a methionine-deficient diet consisting of 91 per cent Canada field peas; 6.0 per cent salt mixture patterned after that of G. M. Briggs *et al.* (*J. Biol. Chem.* **148,** *163 (1943)*); 1.8 per cent cottonseed oil; 1.0 per cent vitamin premix in starch; and 0.2 per cent fortified cod liver oil.

A second group of ten chicks received the same diet supplemented with 0.33 per cent of DL-methionine, 0.09 per cent of L-cystine and 0.16 per cent of DL-tryptophan. C. F. Peterson *et al.* (*Poultry Sci.* **23,** *287 (1944)*) have shown methionine to be the limiting amino acid in Canada field peas. Tryptophan was added because R. J. Block and D. Bolling (*"The Amino Acid Composition of Proteins and Foods," Second Ed., Charles C. Thomas, 1951*) suggested the possibility of a borderline level of tryptophan. Following supplementation, the total calculated value of methionine was 0.5 per cent.

A third diet consisted of a commercial starting mash of the following composition: 10 per cent wheat flour middlings; 42.75 per cent yellow corn meal; 5 per cent finely ground oats; 3 per cent alfalfa meal (17 per cent protein); 25 per cent soybean meal (44 per cent protein); 2 per cent meat scraps; 1.5 per cent fish meal; 1 per cent homogenized condensed fish; 3 per cent corn distillers dried solubles; 0.4 per cent riboflavin in supplement; 3 per cent dried whey fortified with riboflavin; 2 per cent dicalcium phosphate; 1 per cent ground limestone; 0.25 per cent iodized salt; plus vitamin D supplement, vitamin B_{12}, antibiotic supplement and manganese sulfate.

After three months of the above diets, the chicks were fed the diets mixed with 1 per cent of the indicator for a period of 30 to 40 hours. In terms of growth, chicks of the amino acid-supplemented groups weighed an average of 1200 g. and compared well

in general appearance with the starter mash group which weighed 1400 g. Chicks on the Canada field peas, unsupplemented with amino acids, weighed only 480 g. Further supplementation of the pea diet with histidine, glycine, leucine, phenylalanine and valine was without beneficial effect on growth.

In all groups of chicks, the contents of the crop had roughly the same composition with respect to nitrogen and chromic oxide as did the food, indicating relatively little digestion, absorption or selective movement of the contents within this organ. In the gizzard, on the other hand, there was a drop in both nitrogen and chromic oxide. Since the gizzard is not an organ of absorption, the drop in nitrogen and chromic oxide probably reflects the selective movement of soluble, partly digested protein and finely dispersed chromic oxide.

In the duodenum, the high nitrogen concentration, lower chromic oxide and negative absorption coefficients all indicate a considerable secretion of protein into the tract, thus masking any possible absorption detectable by this method. This finding agrees with the well-known fact that the pancreas and intestinal mucosa secrete enzymes into the duodenum.

The nitrogen content and, therefore, the enzyme secretion of the duodenal contents of the methionine-deficient group was definitely lower than that of the other groups. However, this had no noticeable influence on protein digestion, since the absorption coefficients further down the tract were highest for this group. E. S. Nasset (*J. Am. Med. Assoc.* **164,** *172 (1957)*) has suggested that there is sufficient secretion of protein into the tract to present an almost uniform pattern of amino acids for absorption and that the presence of food is in some way stimulating to this secretion.

Absorption was first clearly apparent in the portion of the small intestine just below the duodenal loop where the amount of absorption was also greatest. Absorption continued, at decreasing rates, down to the cecum. Contrary to expectations, the apparent absorption coefficients were highest in the methionine-deficient group and lowest in the starter mash controls.

The authors point out that, although the initial assumption had been that a copious secretion of digestive enzymes was a prerequisite for rapid absorption, it is conceivable that the lower secretion itself contributed to the higher absorption simply because it provided less nitrogen to be reabsorbed, especially since the intestinal enzymes might be relatively resistant to breakdown. They suggest that the lower absorption coefficients of the starter mash group at the upper levels in the tract might be due to a lower digestibility of this diet, which consisted of a variety of vegetable and animal proteins, in contrast to the two other diets consisting largely of field peas. Total absorption of nitrogen by the chicks ranged from 83 per cent (starter mash) to 93 per cent (field peas).

THORACIC DUCT IN LIPID ABSORPTION

Thoracic duct ligation does not depress the absorption of from 4 to 10 per cent of dietary lipid from the gastrointestinal tract of the rat.

It is well known that a large portion of absorbed lipid may be transported from the intestine via the lymphatic system (S. Bergström *et al.*, *Biochem. J.* **58,** *600 (1954)*; B. Bloom *et al.*, *J. Biol. Chem.* **184,** *1 (1950)*; B. Borgström, *Acta physiol. Scandinav.* **25,** *291 (1952)*; *Nutrition Reviews* **10,** *193 (1952)*). However, how essential this transport pathway is for the absorption of normal amounts of dietary lipid remains

an unanswered question. In an effort to provide an answer, J. H. Annegers (*Am. J. Physiol.* **196,** *363* (*1959*)) has studied the effect of thoracic duct ligation on lipid absorption in the rat.

Two types of experiments were performed. In chronic experiments adult rats were fed for one week on a commercial diet containing 4 per cent of lipid, or on the same diet with 10 per cent of oleic acid or olive oil added. Feces were collected and total lipid intake and fecal lipid output were measured. At the end of the week the thoracic duct of each rat was ligated. Then, during successive three-day periods, lipid intake and output were again measured to determine the effect of the ligation on lipid absorption.

The acute experiments were performed on rats that had been starved for 24 hours. The animals were anesthetized, their abdomens were opened and, using a hypodermic syringe, 2 ml. of either oleic acid or olive oil were injected into the duodenum. At this time, the thoracic ducts of a portion of the rats were ligated. Control and experimental animals were killed after six or 18 hours and the amount of lipid remaining in the gastrointestinal tract of each animal was measured.

In only one case in the chronic experiments did lipid excretion after thoracic duct ligation exceed that prior to ligation, and in no case did the average lipid excretion during the ten-day experimental period exceed that during the control period. With 4 per cent of lipid in the diet the average lipid intake and excretion were, respectively, 0.74 and 0.16 g. per day during the pre-operative period, and 0.68 and 0.14 g. per day during the post-operative period. The comparable values for rats fed the basal diet supplemented with 10 per cent of oleic acid were 1.52 and 0.18 g. per day before, and 0.91 and 0.14 g. per day after the ligation. For rats fed the diet containing 10 per cent of olive oil, lipid intake was 1.45 g. and excretion 0.22 g. per day before the operation and 1.82 and 0.30 g. per day, respectively, after the operation.

Fecal excretion of lipid appeared to be a function of intake, so the author performed an analysis of covariance to correct for differences in intake. When this was done, there were no statistically significant differences between the values for lipid absorption obtained before ligation of the thoracic duct and those obtained after ligation.

In the acute experiments, relatively little lipid was found to leave the gastrointestinal tract within six hours after the animals had been given 1.8 g. of either oleic acid or olive oil. The values for the amount of lipid remaining in the intestine after 18 hours were as low as those obtained for fasted rats. Ligation of the thoracic duct was without effect on any of these values.

The logical conclusion from these simple and direct experiments is that thoracic duct transport is not essential in the rat for the absorption of normal amounts of lipid, fed either as free fatty acids or as the glycerides of olive oil. It remains to be determined whether the entire transport of lipid is, under these conditions, shifted to the portal system.

VITAMIN B_{12} AND CARBON TETRACHLORIDE POISONING

Recent work suggests that large doses of vitamin B_{12} may not have any protective effect on the liver when rats are injected with carbon tetrachloride.

Work by H. Popper and his associates suggested that vitamin B_{12} would ameliorate the hepatic necrosis produced in rats by an intraperitoneal injection of carbon tetrachloride (*Nutrition Reviews* **9,** *133* (*1951*)). The protective effect was manifested by an improvement in the bromsulphalein test and a reduction in the

abnormally high weight and fat content of the liver as well as by maintenance of nearly normal liver cell structure. But this was seen only when at least 15 micrograms of vitamin B_{12} per 100 g. of body weight were injected each day just prior to the injection of the carbon tetrachloride. Furthermore, it was reported that when vitamin B_{12} was given a few hours prior to the carbon tetrachloride, the dose of the halogen that could be tolerated by the rat was increased.

K. Aterman and D. Darlington (*Brit. J. Nutrition* **13,** *168 (1959)*) re-investigated the protective effect of vitamin B_{12} in carbon tetrachloride poisoning. They worked with male rats ranging in weight from 105 to 205 g., using litter mates to compensate for the spread in body weights.

The rats receiving one or more injections of 0.15 ml. of carbon tetrachloride per 100 g. of body weight showed fatty changes and loss of nucleic acids and of glycogen, demonstrated by histological staining techniques. Yet these rats had been fed an adequate ration of natural foods and given four daily injections of a vitamin B_{12} concentrate at levels of 10 to 30 micrograms of the vitamin per 100 g. of body weight, far more than the rat's daily requirement. Furthermore, there was no indication of any vitamin deficiency in the animals.

Unfortunately, the investigators did not state the interval elapsing between the time when the carbon tetrachloride was injected and the time when the animals were sacrificed. Their results do show, however, that the injection of vitamin B_{12} had no influence on either the level of fat or on the histology of the livers of the rats injected with carbon tetrachloride.

When the dose of carbon tetrachloride was increased by repeated injections, again there was no difference between the controls and the vitamin-supplemented rats insofar as liver fat levels were concerned. In another experiment, they found that when the rats were injected with a lethal dose of carbon tetrachloride (0.4 ml. per 100 g. of body weight), three out of 20 rats in the control group and two out of 20 in the vitamin-supplemented group survived for more than forty-eight hours.

Thus these observations suggest that large doses of vitamin B_{12} injected just prior to the carbon tetrachloride had no protective action, with respect to liver damage or survival after lethal doses.

Aterman and Darlington (*loc. cit.*) also studied the response of rats maintained on a protein-deficient diet prior to the injection of the carbon tetrachloride. Their reason for carrying out this phase of the study was a statement by H. Popper, D. Koch-Weser and P. B. Szanto (*Proc. Soc. Exp. Biol. Med.* **71,** *688 (1949)*) that the inhibitory effect of vitamin B_{12} was the result of a sparing action of the vitamin on lipotropic agents such as choline. These latter workers claimed that the lipotropic effect is seen only in animals fed a protein-deficient diet, but since the rats with which they had worked had been on a good diet, this explanation could not hold.

When Aterman and Darlington injected carbon tetrachloride into rats fed the protein-free diet for seven days, they found that the 12 animals receiving the vitamin B_{12} supplement had livers containing 6.0 per cent of fat, whereas the livers of controls receiving the carbon tetrachloride alone had 6.8 per cent of fat. However the reduction in liver fat in the vitamin-supplemented animals was significant only at the 5 per cent level. Moreover, histological examination of the livers from the two groups of animals revealed that the improvement associated with the vitamin B_{12} prophylaxis in the above experiment was not apparent in the glycogen depletion of the liver or in the typical "hydropic" or "ballooning" of the liver cells resulting from carbon tetrachloride poisoning.

These investigators felt that the slightly beneficial effect of the vitamin B_{12} was probably attributable to the lipotropic

action of that vitamin. To test this hypothesis, they injected the same amount of vitamin B_{12} used in the previous studies into animals that had been maintained on the protein-free diet for seven days but had received no carbon tetrachloride. Under these circumstances, the liver fat level in the animals injected with vitamin B_{12} was lower than that in the controls (4.4 versus 5.0 per cent). Since the reduction in liver fat level resulting from vitamin B_{12} therapy was the same whether carbon tetrachloride was or was not given, these workers concluded that the effect was in no way related to the hepatotoxic agent.

Although the above evidence appears fairly conclusive, it may be well to postpone a final decision on the possible role of large doses of vitamin B_{12} in liver injury produced by carbon tetrachloride. The work of D. K. Kasbekar, D. V. Rege and A. Sreenivasan (*Nature* **178,** *989* (*1956*)) presents reason for advocating such a delay. These scientists injected 10 micrograms of vitamin B_{12} into each of eight rats (body weights 100 to 150 g.) three hours prior to injecting carbon tetrachloride (2 ml. per kg. of body weight). A comparable group of eight control rats received only the carbon tetrachloride. All rats had been maintained on a stock diet.

The four rats from each of the above groups which were killed six hours after the carbon tetrachloride injection showed similar liver fat levels, 2.8 per cent and 2.9 per cent for the vitamin B_{12} supplemented and control rats respectively. In both groups, the liver fat levels were slightly higher than in the animals which received no carbon tetrachloride (2.3 per cent).

The vitamin B_{12} injected animals which were killed 48 hours after the injection of carbon tetrachloride had lower liver lipid levels than those that did not receive the vitamin (3.5 per cent versus 4.9 per cent). The difference in lipid levels was partly due to the phospholipids which, at the forty-eighth hour, were normal in the vitamin B_{12} injected rats (1.5 per cent) but were reduced in the non-supplemented rats (1.1 per cent).

In addition to the difference in liver lipid levels, the rats receiving the vitamin B_{12} maintained the concentration of ribonucleic acid in their livers (0.9 per cent and 1.0 per cent at six and 48 hours respectively). On the other hand, the non-supplemented rats showed a slight reduction in ribonucleic acid levels (0.9 per cent and 0.7 per cent). A similar situation existed insofar as glutathione levels in the liver were concerned. The vitamin B_{12} supplemented rats had 0.13 per cent and 0.17 per cent of glutathione at six and 48 hours respectively after the carbon tetrachloride injection, while the non-supplemented rats had levels of 0.04 per cent and 0.12 per cent at similar intervals.

Vitamin B_{12} supplementation had no influence on the glycogen levels of the livers as determined by chemical analysis. Also, no differences throughout the experiment were seen in the liver levels of deoxyribonucleic acid and methionine.

An extension of the above work by D. K. Kasbekar and A. Sreenivasan (*Nature* **178,** *990* (*1956*)) suggests that the mitochondria in the livers of rats injected with vitamin B_{12} three hours prior to the injection of carbon tetrachloride were more nearly normal than those from rats not injected with the vitamin.

The mitochondria from the livers were incubated in a phosphate buffer, either alone or with the addition of magnesium chloride, adenosine monophosphate and glutamate to maintain conditions for oxidative phosphorylation. At various intervals, samples were centrifuged at high speed to bring down the larger particles. After deproteinizing the supernatant, the optical density at 260 millimicrons indicated much less absorptive material released by the mitochondria from the animals that had not received any vitamin B_{12}. This was true for both the mitochondria incubated alone in

the phosphate buffer and the preparations incubated with the compounds maintaining oxidative phosphorylation.

The above data is interpreted by Kasbekar and Sreenivasan to mean that liver cells injured by carbon tetrachloride have mitochondria that show a reduced rate of release of their nucleotides (as measured by absorption at 260 millimicrons). When vitamin B_{12} was injected into the animals three hours prior to the carbon tetrachloride, the vitamin appeared to maintain the mitochondria in a condition more nearly like that of the normal animal which had received no carbon tetrachloride.

Further reason for postponing a decision concerning the role of vitamin B_{12} lies in the work of R. S. Yamamoto, K. Okuda and B. F. Chow (*Proc. Soc. Exp. Biol. Med.* **94,** *497 (1957)*), who found that the injection of carbon tetrachloride into adult rats produced an increase in the serum vitamin B_{12} levels. When 0.035 ml. of carbon tetrachloride per 100 g. of body weight was injected into five 400 g. rats, the vitamin B_{12} plasma levels 24 hours after the injection increased from the control level of 0.66 to 0.87 mg. per ml. The rats receiving twice as much carbon tetrachloride had still higher plasma levels (1.17 mg. per ml.). The plasma levels for all three groups were the same by the seventh day after the carbon tetrachloride injection.

To verify the fact that the increase in the plasma levels was due to true vitamin B_{12}, Yamamoto and co-workers repeated the assays after treating the samples with alkali. The test organism used by them, *Lactobacillis leichmannii*, responds to a number of non-vitamin B_{12} compounds, but the activity of these compounds is destroyed by alkali treatment. The results of this precautionary measure showed that the increase in the plasma levels was due to true vitamin B_{12}.

In conclusion, the apparent contradiction in some of the above work makes it difficult to state whether vitamin B_{12} does or does not have a protective effect on the livers of rats injected with small amounts of carbon tetrachloride. The results secured by Aterman and Darlington, however, offer conclusive evidence that under their conditions, the vitamin has no effect when a lethal dose of carbon tetrachloride is injected into rats. It should be emphasized that in all of these studies, the levels of vitamin B_{12} are in the pharmacologic range and far above the daily nutritional requirements.

MANGANESE-IRON ANTAGONISM

Although as small an amount as 125 p.p.m. of manganese in the food will interfere with hemoglobin synthesis, addition of extra iron overcomes this effect.

It was demonstrated by R. H. Hartman *et al.* (*J. Nutrition* **57,** *429 (1955)*) that 2000 p.p.m. of manganese in the diet interferes with regeneration of hemoglobin in lambs. The question was then raised as to the possibility of overcoming the manganese effect by adding iron to the diet. Also in need of investigation was the minimal level of manganese in the diet which would interfere with hemoglobin regeneration. G. Matrone, R. H. Hartman and A. J. Clawson (*J. Nutrition* **67,** *309 (1959)*) have investigated these problems using rabbits and baby pigs.

In all, four experiments were carried out, one with rabbits and three with baby pigs. The first was designed to study the effect of excessive manganese on hemoglobin regeneration of rabbits, while the second sought to determine the effect of excessive manganese and of additional iron on the hemoglobin regeneration of baby pigs. The minimal levels of dietary manganese that will retard hemoglobin regeneration in

baby pigs were studied in the third and fourth experiments. Both the rabbits and pigs were rendered anemic by a diet of cow's milk.

Addition of 2000 p.p.m. of manganese to the regeneration diet of rabbits resulted in a retardation of hemoglobin regeneration which was, however, overcome by the end of the six-week experimental period. The greatest depressing effect of manganese on hemoglobin regeneration was observed at about the second week.

Hemoglobin regeneration in baby pigs was also depressed by 2000 p.p.m. of manganese, but the manganese effect was overcome by addition of 400 p.p.m. of iron. When two pigs which had been on a relatively low iron supplement (25 p.p.m.) for 12 days were transferred to a diet containing 400 p.p.m. of iron, they regenerated hemoglobin at such a rapid rate that by 21 days their hemoglobin values were similar to those fed diets with either 400 p.p.m. of iron or with no supplemental manganese.

When the pigs receiving zero, 125, 250 and 2000 p.p.m. of manganese were studied with respect to weight gain, it was found that the highest average gains were made by the pigs with no manganese and the lowest by the pigs on the 2000 p.p.m. level of manganese. The increases in hemoglobin levels of the pigs on the 125 and 500 p.p.m. manganese diets were significantly lower than were those on the zero level, and the pigs on the 2000 p.p.m. level synthesized a significantly smaller quantity of hemoglobin than did those on the 125 or 500 p.p.m. levels. The overall response to manganese followed a sigmoid curve with a sharp drop in the hemoglobin increment between the first and second levels of manganese, little change between the second and third levels and a sharp drop between the third and fourth levels of manganese.

In the fourth experiment it was found that the hemoglobin increment made by pigs receiving 50 p.p.m. of manganese was not significantly lower than that of those on the zero level.

Comparing the two species, it was observed that hemoglobin regeneration of the rabbits on the high level of manganese was faster than that of the baby pigs and eventually even approached a normal value. The authors assume that the anemic baby pigs required iron not only for hemoglobin repletion but also for hemoglobin increases associated with growth, whereas the mature anemic rabbits need iron primarily for hemoglobin regeneration.

It is possible that the effect on growth in the pigs was an indirect result of the anemia rather than a direct effect of manganese on the rate of weight gain. Hartman *et al.* (*loc. cit.*) did not observe such a depression on growth rate. However, it is also possible that enzymes involved in protein and other syntheses require a certain level of iron in order to be fully effective.

These results, which show that additional iron in the diet can overcome the depressing effect of manganese, reveal a similarity between plants and animals concerning the effect of toxic levels of manganese on the metabolism of iron. I. I. Somers and J. M. Shive (*Plant Physiol.* **17,** *582 (1942)*) have shown that toxic levels of manganese bring about an iron chlorosis in plants which can, however, be overcome by additional iron.

NOTES

Plasma Unesterified Fatty Acids and Glycerides Following Surgery

The role of unesterified fatty acids in the general economy of the body has been reviewed earlier (*Nutrition Reviews* **14,** *216 (1956)*; **16,** *135 (1958)*). Elevated plasma levels have been noted in those conditions where the body has an increased metabolism of tissue fat.

Recently, L. B. Wadström (*Acta chir. Scandinav.* **116,** *167 (1959)*) has studied the changes in the unesterified fatty acid concentration of plasma of patients before and following surgery. He also studied the glyceride and phospholipid levels under the same conditions. Two groups of patients were studied, the first consisting of seven individuals (four women and three men) who underwent a cholecystectomy, and the second consisting of five men having a repair of a hernia.

The patients undergoing a cholecystectomy were studied for four days preoperatively, during which time they were maintained on a nutritional regimen simulating the one they would receive postoperatively. In this manner, it was possible to separate the effects caused by nutrition from those produced by trauma.

On the first control day, which resembled the day of operation, the patients received intravenously 110 g. of glucose in 2000 ml. of water. They were allowed free access to water but received no food orally. On the second control day, which simulated the first postoperative day, they received intravenously 55 g. of glucose in 1000 ml. of water plus 1000 ml. of a solution containing sodium and potassium chloride. Oral food was again prohibited. On days three and four the patients were allowed to eat the regular hospital meals and were up most of the time.

The operation was performed on the fifth day and the patients' nutrition on this and the first postoperative day was provided by intravenous infusions as described for the control period. From the seventh study day (second postoperative day) on, the patients ate the regular hospital meals, although actual food consumption was not recorded.

This program of study provided the patient with a similar but restricted caloric intake during the control as well as postoperative period. It was noted that the changes in plasma lipids were, on the whole, the same for both periods. The author indicated that the postoperative changes found in plasma lipids concentration may be the result of the restricted caloric intake consisting only of sugar solutions.

However, a significant increase in the unesterified fatty acid concentration and also a significant decrease in the concentration of the glycerides in the plasma, were noted if the day of operation was compared with its corresponding control day. The increase in the unesterified fatty acid level of the plasma following surgery is believed to result from the increased mobilization of fat from the fat depots.

Changes of a similar type were noted for the patients undergoing a hernia repair, but since no comparable calorie control period was provided, the interpretation of the differences is not as well established.

Prevention of Scurvy by Fortification of Evaporated Milk

The practice of enrichment of foods with vitamins has been notably successful in prevention of specific diseases of deficiency. Addition of vitamin D to milk and thiamine to bread are good examples. With less spectacular results, vitamin A has been added to skim milk, margarine and butter, and other B-complex vitamins have been added to bread and cereals.

Efforts have been made to encourage addition of ascorbic acid to foods such as milk (*Nutrition Reviews* **6,** *255 (1948)*; H. Medovy, *Canad. Med. Assn. J.* **80,** *213*

(*1959*)) and to canned fruits, fruit juices and frozen fish (*Nutrition Reviews* **9,** *1,* (*1951*)). In some instances, the vitamin was suggested as a means of preserving the color or flavor of the food as well as providing desirable fortification.

Before deciding to advise enrichment of a given food with a vitamin, one should answer the following questions. (1) Is enrichment necessary or can this vitamin be obtained easily and inexpensively from natural foods available to the population in question? (2) Would excessive amounts of this vitamin be harmful? (3) Would enrichment adversely alter the appearance, taste or odor of the product? (4) Would the consumer gain a false sense of security which would encourage dietary neglect of other essential nutrients?

D. Grewar (*Canad. Med. Assn. J.* **80,** *977* (*1959*)) reported experiences in Manitoba between 1953 and 1958 with almost 40 cases of infantile scurvy occurring in children of indigent parents, often Indian or Eskimo.

He described in detail three infants who had been fed a standard formula consisting of one part of evaporated milk and one part water sweetened with sugar. Clinical symptoms of scurvy had developed (sometimes coincident with an infection) and the scurvy was confirmed by radiograph.

The cases were treated with a vitamin C fortified, partially skimmed evaporated milk product which had been stored in a warm room for six months prior to use. This prepared formula was autoclaved at 215° for fifteen minutes and, after sterilization, two random samples contained 74 and 94 mg. of ascorbic acid per quart respectively. The three scorbutic infants fed this formula alone, without additional vitamins or other foods, recovered promptly after six days (although two required antibiotic treatment for respiratory infections). The radiologic evidences of scurvy began to subside after two weeks.

The author concluded that, although breast feeding would be most desirable, its popularity has been declining, and the incidence of infantile scurvy has reached epidemic proportions in several areas of Canada. Scurvy occurred for the most part in circumstances of poverty and ignorance where certain geographical locations made availability of fresh foods and vegetables difficult. Consequently, he recommended as standard practice the fortification of all evaporated milk with vitamin C.

While this might be desirable for remote sections of Canada in which some people are poor and uneducated, the chief objection to this practice in the United States would be the price differential. Compulsory fortification of all evaporated milk could be expected to increase the cost and to work additional hardships upon the indigent. Grewar's suggestion might apply to some regions of any country, but not to the United States in general.

Recent Books

Breast Feeding and Artificial Feeding (The Norrbotten Study), by O. Mellander, B. Vahlquist, T. Mellbin and collaborators. *Acta Paediatrica* **48,** *Suppl. 116*. Published by Almquist & Wiksells Boktryckeri AB, Uppsala. Pp. 108.

Nutritional Diagnosis, by Grace Goldsmith. Published by Charles C. Thomas. Pp. 164.

Annual Report 1957–58, The Nutrition Research Laboratories, Coonoor (South India). Indian Council of Medical Research. Published by Cambridge Printing Works, Delhi. Pp. 60.

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